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ORIGINAL RESEARCH

Rapid determination of anastrozole in plasma by gas chromatography with electron capture detection and its application to an oral pharmacokinetic study in healthy volunteers

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ABSTRACT: A rapid, sensitive and accurate capillary gas chromatographic assay with ^{63}Ni electron capture detection was developed for the determination of anastrozole in human plasma. It comprises a one-step liquid–liquid extraction procedure and gas chromatography on a capillary column using constant oven temperature. This method has been applied to the oral pharmacokinetic study of anastrozole in healthy Chinese male volunteers. Pharmacokinetic parameters of two anastrozole preparations were evaluated after single, oral administrations to 18 subjects at a dose of 1 mg in a single-blind cross-over trial. Plasma anastrozole concentration–time profiles were best described by a two-compartment model. After oral administrations of imported and domestic anastrozole tablets, the t_{max} and C_{max} were 1.52 ± 1.04 h and 8.75 ± 3.03 ng/mL for the former, and 1.43 ± 1.12 h and 9.44 ± 3.59 ng/mL for the latter; the elimination half-life was 46.0 ± 25.2 h vs 41.2 ± 8.8 h, and the area under the curve (AUC) was 423 ± 114 ng h/mL vs 444 ± 157 ng h/mL. The result indicates that the two products are bioequivalent. Copyright © 2002 John Wiley & Sons, Ltd.

INTRODUCTION

Anastrozole, [2, 2′-[5-(1H-1, 2, 4-triazole-1-y-methyl)-1, 3-phenylene]bis(2-methylpropiononitrile)], is a novel, selective and potent triazole aromatase inhibitor developed by AstraZeneca Pharmaceutical Co. Ltd. In postmenopausal women, oestraiol is produced primarily from the conversion of androstenedion to oestrone through the aromatase enzyme complex in peripheral tissues. Oestrone is subsequently converted to oestradiol. Anastrozole can effectively suppress the production of oestradiol by binding to the cytochrome P450 part of the enzyme complex and thus blocking NADPH utilization. Anastrozole has been recommended as the first-line treatment for breast cancer in post-menopausal women recently.

To our knowledge, there has been no detailed report about the pharmacokinetic profile of anastrozole yet. Only values of C_{max} and elimination half-time of anastrozole have been reported in a summary of clinical studies (Plourde *et al.*, 1994).

Due to the low dose (1 mg) of anastrozole, the plasma concentration of anastrozole is rather low, which is a difficulty for the detection of plasma anastrozole. There are few reports about the determination of anastrozole in biological samples. High-performance liquid chromatography with ultraviolet, fluoresencence or electrochemical detection is not sensitive enough to detect anstrozole in the plasma from clinical study. A previously reported method for the determination of plasma anastrozole by gas chromatography used a temperature programme (Bock *et al.*, 1997), which makes the analytical procedure quite time-consuming, especially when a large quantity of samples is to be analysed. Good resolution between anastrozole and dezapam (internal standard) or endogenous substances was achieved under a constant oven temperature after a one-step liquid–liquid extraction procedure in this study.

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Abbreviations used: AIC, Alkaike's information criterion; AUC, area under the curve; C_p, plasma concentration; C_{max}, peak concentration; Cl, clearance; t_{max}, time to achieve maximum concentration; V_c, volume of central compartment.

EXPERIMENTAL

Instrumentation and conditions. The HP 6890 series GC system (Angilent, Wilmington, DE, USA) consisted of an automatic sampler, an oven, a ^{63}Ni electron capture detector and a HP chemstation. Separation was achieved using a HP-50 fused-silica capillary column (cross-linked 50% phenyl-methyl silicone,

30 m × 530 μm i.d., 1 μm film thickness; Angilent, Wilmington, DE, USA). The temperature of inlet, oven and detector was set at 260, 230 and 260°C, respectively. Both carrier gas and make-up gas were nitrogen. The column pressure was 200 kPa. The flow rate of carrier gas was 48.8 mL/min and that of the make-up flow 45 mL/min.

Chemicals and reagents. Anastrozole standard was supplied by Wanma Pharma Co. Ltd. Diazampin (internal standard, I.S., purity >99.5%) was obtained from Shanghai Institute for Drug Control. Stock solutions of anastrozole and diazampin were both prepared in ethyl acetate at a concentration of 1 μg/mL. All other chemical reagents were of analytical grade, purchased from Shanghai Chemical Reagent Co. Ltd. Ethyl ether and ethyl acetate were distilled before use.

Two anastrozole formulations (A and B) of 1 mg tablets were used for comparative pharmacokinetic study. Formulation A was Arimidex tablets (patch no. OA 1845A) produced by AstraZeneca Pharmaceutical Co. Ltd (Macclesfield, UK), and formulation B (batch no. 000102) supplied by Wanma Pharma Co. Ltd (Hangzhou, China).

Extraction procedure. To 1 mL plasma placed in a 15 mL glass tube, 100 μL of diazampin solution (1 μg/mL) and 1 drop of ammonia water were added. The samples were extracted with 7 mL ethyl ether for 2 min on a vertex mixer. After stationary extraction, the organic layers were transferred into another clean tube and evaporated to dryness under a nitrogen stream with a water bath (40°C). The residues were dissolved in 150 μL ethyl acetate. Aliquots of 5 μL were injected into the GC system.

Calibration. Calibration curves were prepared with blank plasma samples spiked with anastrozole to cover the concentration range from 0.5 to 200.0 ng/mL and with the internal standard at the fixed concentration of 100 ng/mL. Calibration graphs were obtained by plotting drug concentrations against the peak–area ratio of anastrozole–diazampin. The concentrations of unknown samples (anastrozole) were determined using the linear regression line (unweighed) of the calibration standard.

Method validation. The accuracy of the analytical method was determined by comparing the concentrations of anastrozole found from plasma with the calibration method to the theoretical concentrations of anastrozole (1.0, 10.0 and 20.0 ng/mL, five replicates at every concentration). The extraction recovery of anastrozole in human plasma was determined in triplicate at concentrations of 1.0, 10.0 and 20.0 ng/mL by comparing the data obtained by the direct injection of standard aqueous solutions to those obtained after the whole extraction procedure. Inter-day and intra-day precision of the method were evaluated by analysing drug-free plasma to which anastrozole had been added at concentrations of 1.0, 10.0 and 20.0 ng/mL, in five replicates.

Pharmacokinetic study. Eighteen healthy Chinese male human volunteers were selected for the study after giving written informed consent and having normal biochemical parameters. Subjects were aged 22.5 ± 1.0 years (mean ± SD, range 21–24), with a mean body weight of 66.4 ± 7.3 kg (range 56.5–80.1).

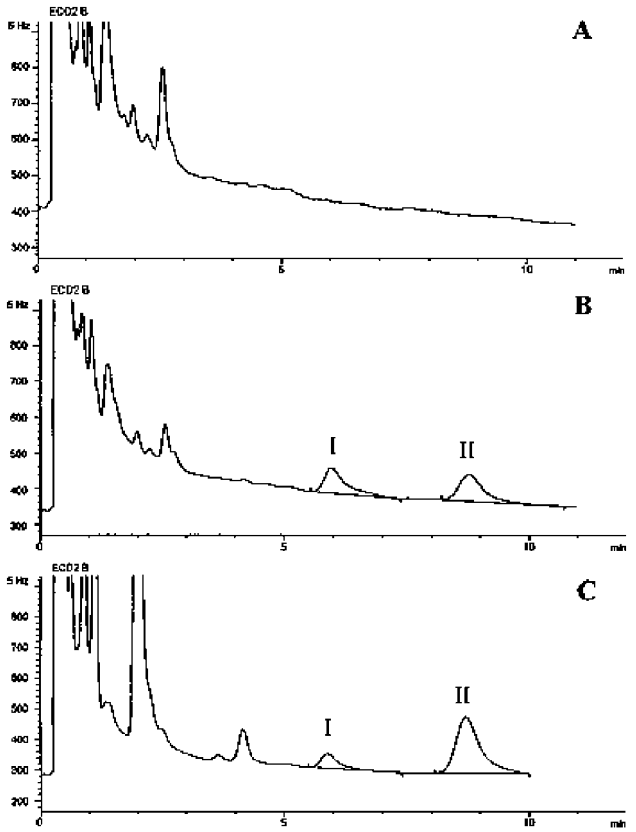

Figure 1. Chromatograms of (A) blank plasma, (B) plasma spiked with anastrozole and dezapam and (C) plasma sample: **I**, anastrozole; **II**, I.S.

Two formulations of anstrozole tablets (1 mg, product A and product B) were studied. One tablet of either brand was administered to each volunteer with 200 mL of water, such that half of the number of volunteers received product A and the rest received product B in the first part of a single-blind cross-over study. The second part of the study was carried out 14 days later. Identical conditions were maintained on both occasions.

Blood samples, obtained from an antecubetal vein prior to dosing and at 0.33, 0.66, 1.0, 1.5, 2.0, 3.0, 5.0, 10.0, 26.0, 58.0, 82.0 and 106.0 h after dosing were placed in heparinized tubes. The samples were immediately centrifuged at 3000***g*** for 15 min, and the plasma samples were separated and frozen at −20°C until analysis.

Curve fitting and pharmacokinetic parameters calculation were carried out by estimation using the Practical Pharmacokinetic Program (3p97 program, edited and published by Chinese Pharmacological Association). An appropriate pharmcokinetic model was chosen on the basis of lowest weighed squared residuals, lowest Alkaike's information criteion (AIC) value, *r*-squared and correlation coefficient. The area under the curve (AUC) was calculated by trapezoidal rule between 0 h and the last sampling time plus C_p/β, where C_p is the plasma concentration of the last sampling and β is the elimination rate constant. The time (t_{max}) taken to achieve peak concentration (C_{max}) was calculated using differential calculus. The results are presented as means and standard errors of mean.

Table 1. Precision and accuracy of GC-ECD determination in plasma

Concentration added (ng/ml)	Concentration detected (mean ± SD, $n = 5$; ng/ml)	RSD (%)	Accuracy (%)
Inter-day ($n = 5$)			
1.0	0.935 ± 0.045	4.8	93.5
10.0	10.02 ± 0.39	3.9	100.2
20.0	19.80 ± 0.34	1.7	99.0
Intra-day ($n = 5$)			
1.0	0.891 ± 0.078	8.8	89.1
10.0	9.55 ± 0.28	2.9	95.5
20.0	18.84 ± 0.79	4.2	94.2

RESULTS AND DISCUSSION

Analysis of anastrozole

The retention time of anastrozole and internal standard under the described gas chromatographic conditions were found to be 5.9 and 8.8 min, and no interfering peaks were detected in the blank plasma or in plasma samples. Figure 1 shows typical chromatograms of extracts from blank plasma, blank plasma spiked with anastrozole and internal standard and plasma sample from a volunteer.

A linear relationship was found between the peak area ratios of anastrozole to diazampin and plasma anastrozole concentration over the range of 0.5–200 ng/mL with a correlation coefficient of 0.9997 ($n = 9$). It confirmed the equation $y = 0.01803C_p - 0.03144$, where y refers to the area ratio of anastrozole to diazampin. The low detection limit of anastrozole was 0.2 ng/mL of plasma was defined as a signal-to-noise ratio greater than 3.

Precision and accuracy studies in plasma showed an acceptable relative standard deviation (<10%) and high accuracy for both inter-day ($n = 5$) and intra-day ($n = 5$) studies (<10%), as shown in Table 1. The extraction recoveries (absolute recovery) of anastrozole were between 84.5 and 97.0%, dependent on the plasma anastrozole concentration.

Pharmacokinetic parameters

The mean plasma concentration–time curve of anastrozole over 106 h after an oral administration is presented in Fig. 2. The mean plasma concentration of anastrozole was best fitted to a linear two-compartment open model. Various pharmacokinetic parameters, including absorption (α) and elimination (β) rate constants, elimination half-life ($T_{1/2\beta}$), volume of central compartment (V_c), maximal plasma concentration (C_{max}), the time to achieve maximal plasma concentration (t_{max}), clearance (Cl) and AUC, were calculated for individual volunteers.

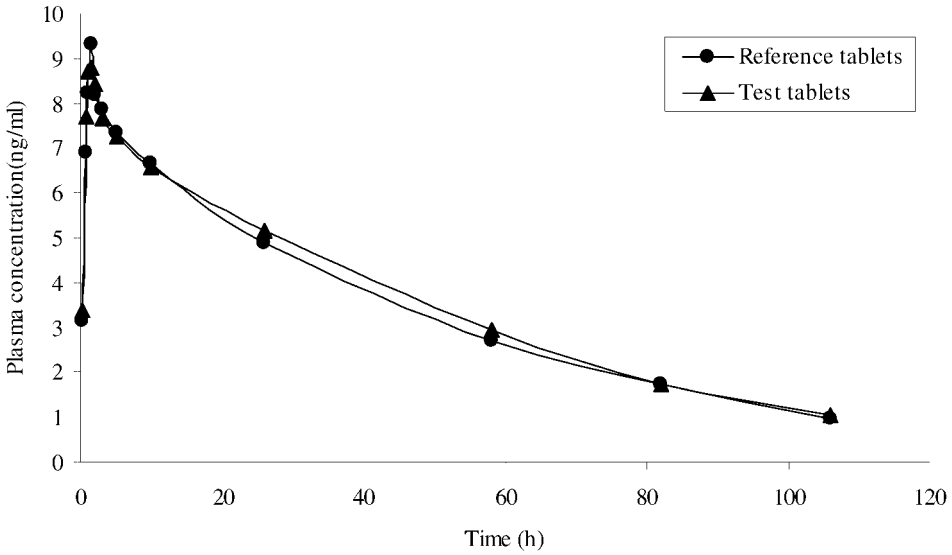

Figure 2. Mean plasma concentration-time curves of anastrozole after p.o. tested tablets, and reference tablets ($n = 12$).

Table 2. Pharmacokinetic parameters following oral administration of anastrozole test tablet and reference tablet ($n = 18$)

Parameter	Test tablet	Reference tablet
A (ng/mL)	14.0 ± 11.6	9.33 ± 9.13
α (L/h)	1.24 ± 1.00	0.907 ± 0.739
B (ng/mL)	7.44 ± 2.44	7.16 ± 3.24
β (L/h)	0.0176 ± 0.0038	0.0171 ± 0.0044
Lag time (h)	0.192 ± 0.104	0.198 ± 0.113
$t_{1/2(\beta)}$ (h)	41.2 ± 8.8	46.0 ± 25.2
k_{21} (L/h)	0.727 ± 0.549	0.634 ± 0.612
k_{10} (L/h)	0.0312 ± 0.0110	0.0277 ± 0.0074
k_{12} (L/h)	0.516 ± 0.453	0.261 ± 0.262
V_c (L)	0.117 ± 0.117	0.0965 ± 0.0336
CLs (L/h)	0.00233 ± 0.00085	0.00252 ± 0.00063
t_{max} (h)	1.43 ± 1.12	1.52 ± 1.04
C_{max} (ng/mL)	9.44 ± 3.59	8.75 ± 3.03
AUC (h ng/mL)	444 ± 157	423 ± 114

The means of the above parameters for a group of 18 volunteers are presented in Table 2.

Following oral administration of anastrozole in subjects, the drug reached a peak concentration of 8.75 ng/mL (or 9.44 ng/mL) in the blood within 1.6 h. The elimination half-life (46.0 or 41.2 h) of anastrozole is consistent with the reported value (38–61 h; Plourde *et al.*, 1994), while the peak plasma concentration (8.75 or 9.44 ng/mL) is approximately two-thirds of reported value (13.1 ng/mL; Plourde *et al.*, 1994).

The mean C_{max} and the mean AUC for both preparations were comparable and were not significantly different from each other ($p < 0.05$). The t_{max} for both preparations was between 1.4 and 1.6 h. Individual differences exist in most pharmacokinetic parameters ($p > 0.05$), especially in the absorption stage. The result indicates that the pharmacokinetic profiles of the two formulations are identical and the bioavailability is not significantly different at $p < 0.05$, indicating the bioequivalence of the two products.

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